Exhibit B

In order to analyze structural and functional features of such receptor molecules monoclonal antibodies (mAb) to glucocorticoid receptor of rat liver were generated. Spleen cells from Balb/c mice immunized with partially purified native receptor from rat liver cytosol were fused with the mouse myeloma cell line X63 Ag8.653. Out of 102 hybrids obtained 76 secreted immunoglobulin. Hybridoma supernatants were screened by immunoprecipitation of the [^{3}H] steroid-receptor complex using rabbit antimouse IgG coupled to Sepharose 4B. 8 positive cultures were identified, 4 of which were cloned by limiting dilution and subsequently mAb were produced in ascites. The interaction of these mAb with the glucocorticoid receptor was analyzed by sucrose-density gradient centrifugation and measurement of avidity by titration. Cross-reactivity with glucocorticoid receptors from other tissues of the rat and from other species was determined.

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115. Monoclonal antibodies against the fifth component of human complement

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Although the biological effects of C5 are well understood, the molecular basis for these effects is still controversial. Due to their inherent high specificity for one epitope on the antigenic molecule, monoclonal antibodies are ideal tools to investigate biological functions. The somatic cell fusion technique was used to obtain monospecific antibodies directed against C5 of human complement.

Intact human C5 was isolated from fresh plasma by the method of KUNKEL et al. (1980) as modified by DESSAUER and ROTHER (1982). The C5 preparation provided high purity as determined by rocker and crossed immunoelectrophoresis and was highly active in hemolysis.

DBA/2 mice were immunized intraperitoneally with 100 µg of purified C5 in complete Freund's adjuvant, followed 4 weeks later with 50 µg C5 in incomplete Freund's adjuvant. Three days prior to cell fusion 10 µg C5 was injected intravenously. For cell fusion 5×10^7 P3-X63-Ag8 myeloma cells and 10^8 spleen cells of the immunized mouse were exposed to 50 % polyethylene glycol 4000 for 2 minutes at 37 °C. Hybrids were selected in HAT-medium. Antibody-secreting hybrid cells were detected by a solid-phase radioimmunoassay with the antigen immobilized on polyvinylchloride plates. To achieve monoclonality the limiting dilution technique was employed. For mass production of antibodies the hybrid cells were injected into pristane primed CD2F1/Han (Balb/c X DBA/2) F1 mice.

Culture supernatants from 30 hybridoma cells were found to contain anti C5 antibodies. 10 hybridoma antibodies were tested for inhibition of C5a effects using a serotonin release assay with guinea pig platelets as target cells. Two of these antibodies demonstrated significant inhibition.

This result showed high specificity of monoclonal anti C5 antibodies for one antigenic determinant only. Therefore it will be possible to study functional properties of C5 by well-defined monospecific antibodies.